REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
	New Reprint	-

4. TITLE AND SUBTITLE

Plant virus directed fabrication of nanoscale materials and devices

5a. CONTRACT NUMBER
W911NF-14-1-0286
5b. GRANT NUMBER
5c. PROGRAM ELEMENT NUMBER
611102
5d. PROJECT NUMBER
5e. TASK NUMBER
5f. WORK UNIT NUMBER

6. AUTHORS

James N. Culver, Adam D. Brown, Faheng Zang, Markus Gnerlich, Konstantinos Gerasopoulos, Reza Ghodssi

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

University of Maryland - College Park
Office of Research Administration
3112 Lee Building
College Park, MD 20742 -5141

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
65392-LS.1

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

Bottom-up self-assembly methods in which individual molecular components self-organize to form functional nanoscale patterns are of long-standing interest in the field of materials sciences. Such self-assembly processes are the hallmark of biology where complex macromolecules with defined functions assemble from smaller molecular components. In particular, plant virus-derived nanoparticles (PVNs) have drawn considerable attention for their unique self-assembly architectures and functionalities that can be harnessed to produce new materials for industrial and biomedical applications. In particular, PVNs provide simple systems to model and assemble nanoscale particles

15. SUBJECT TERMS

Nanotechnology; Bio-materials; Virus particles; Virus assembly; Virus-like particles

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			James Culver
UU	UU	UU	UU		19b. TELEPHONE NUMBER
					301-405-2912

Standard Form 298 (Rev 8/98)
Prescribed by ANSI Std. Z39.18

Report Title

Plant virus directed fabrication of nanoscale materials and devices

ABSTRACT

Bottom-up self-assembly methods in which individual molecular components self-organize to form functional nanoscale patterns are of long-standing interest in the field of materials sciences. Such self-assembly processes are the hallmark of biology where complex macromolecules with defined functions assemble from smaller molecular components. In particular, plant virus-derived nanoparticles (PVNs) have drawn considerable attention for their unique self-assembly architectures and functionalities that can be harnessed to produce new materials for industrial and biomedical applications. In particular, PVNs provide simple systems to model and assemble nanoscale particles of uniform size and shape that can be modified through molecularly defined chemical and genetic alterations. Furthermore, PVNs bring the added potential to “farm” such bio-nanomaterials on an industrial scale, providing a renewable and environmentally sustainable means for the production of nano-materials. This review outlines the fabrication and application of several PVNs for a range of uses that include energy storage, catalysis, and threat detection.

REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

Continuation for Block 13

ARO Report Number 65392.1-LS
Plant virus directed fabrication of nanoscale mat...

Block 13: Supplementary Note

Approved for public release; distribution is unlimited.

Virology 479-480 (2015) 200–212

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Plant virus directed fabrication of nanoscale materials and devices

James N. Culver [a,b,*], Adam D. Brown [a,c], Faheng Zang [d,e], Markus Gnerlich [e], Konstantinos Gerasopoulos [d,f], Reza Ghodssi [d,e,f]

[a] Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD 20742, USA
[b] Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA
[c] Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA
[d] Institute for Systems Research, University of Maryland, College Park, MD 20742, USA
[e] Department of Electrical and Computer Engineering, University of Maryland, College Park, MD 20742, USA
[f] MEMS Sensors and Actuators Laboratory, University of Maryland, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 18 December 2014
Returned to author for revisions
24 February 2015
Accepted 2 March 2015
Available online 26 March 2015

Keywords:
Nanotechnology
Bio-materials
Virus particles
Virus assembly
Virus-like particles

ABSTRACT

Bottom-up self-assembly methods in which individual molecular components self-organize to form functional nanoscale patterns are of long-standing interest in the field of materials sciences. Such self-assembly processes are the hallmark of biology where complex macromolecules with defined functions assemble from smaller molecular components. In particular, plant virus-derived nanoparticles (PVNs) have drawn considerable attention for their unique self-assembly architectures and functionalities that can be harnessed to produce new materials for industrial and biomedical applications. In particular, PVNs provide simple systems to model and assemble nanoscale particles of uniform size and shape that can be modified through molecularly defined chemical and genetic alterations. Furthermore, PVNs bring the added potential to "farm" such bio-nanomaterials on an industrial scale, providing a renewable and environmentally sustainable means for the production of nano-materials. This review outlines the fabrication and application of several PVNs for a range of uses that include energy storage, catalysis, and threat detection.

Contents

* Correspondence to: University of Maryland, College Park, MD 20742.
Tel.: +1 301 405 2912; fax: +1 301 314 9075.
E-mail address: jculver@umd.edu (J.N. Culver).

http://dx.doi.org/10.1016/j.virol.2015.03.008
0042-6822/

Introduction

Advances in nanotechnology offer significant improvements in a wide range of applications that include light weight materials with greater strength, increased energy efficiency from electronic devices, and better sensors for a range of environmental, pharmaceutical and manufacturing uses. A key challenge to achieving these improvements is the ability to assemble and pattern diverse components into functioning nanoscale devices. Current "top-down" semiconductor processing techniques such as lithography have limitations with respect to the scale of assembly, complexity of the desired nanofeatures and cost of synthesis (Fan et al., 2013). In comparison, biological systems function almost exclusively through the molecular assembly of diverse components to produce molecular machines of incredible complexity. Microtubule kinesin and bacterial flagella motors are two examples of the types of "bottom up" self-assembly systems that are sought by engineers yet routinely produced at the nanoscale within biological systems (Browne and Feringa, 2006; Korten et al., 2010; Stock et al., 2012). However, the development of such sophisticated assembly systems requires new engineering advances that integrate or mimic these "bottom-up" self-assembly methods.

To achieve these advances scientists from biological and engineering disciplines have investigated the nanoscale structures of viruses as simple systems from which to develop design, synthesis and application strategies for the production and functionalization of self-assembling materials. Viruses have evolved exquisite macromolecular particles in which to store and protect their genomes. These particles also play critical roles in cell entry, virus movement and in many instances virus replication. The functional demands placed upon these particles has resulted in the development of extremely uniform and complex molecular structures that are derived from the self-assembly of a limited number of molecular building blocks. In fact, virus particles represent ideal nanomachines that not only self-assemble into macromolecules of defined shape and size but also function as environmental sensors for the controlled release of their genomes upon cell entry. The same virus particles also operate as information storage devices that carry genetic instructions to produce the next generation of components required for self-assembly. These combined features have led to the incorporation of virus structures into an array of devices and applications that have broken new ground in the development of biomaterials (Douglas and Young, 1998; Shenton et al., 1999; Whaley et al., 2000). In particular, plant viruses have received significant interest as both models and materials for the establishment of strategies that combine biological processes with traditional top-down manufacturing systems. Characteristics that make many plant viruses attractive for these studies are their relative simplicity, including the lack of membranes, ease of purification and simple one or two protein capsid assemblies that are structurally well defined. In addition, genetic programmability and in vitro assembly systems permit the uniform production of particles with designer functionalities such as antigen display and analyte recognition. In this review we primarily focus on the application of plant virus-derived nanoparticles (PVN) as nanoscale scaffolds and containers for the development of materials and devices with unique functionalities. However, space constraints limit our ability to fully cover the diverse array of PVN applications currently being investigated. In addition, we have not attempted to fully address the considerable work being done in the application of PVN in vaccine development, bio-imaging and drug delivery. To address these deficiencies we recommend several additional reviews that cover the application of viruses and protein structures in nanotechnology and biomedicine applications (Alonso et al., 2013a; Fan et al., 2013; Franzen and Lommel, 2009; Li and Wang, 2014; Lomonossoff and Evans, 2014; Rong et al., 2011; Young et al., 2008).

PVN characteristics for biomaterial fabrication

Several features make plant viruses useful as scaffolds for the synthesis of nano-materials. First, their simple virions derived from the self-assembly of defined protein subunits are remarkably stable and are of uniform size and shape. The repeating patterns of amino acid side chains displayed by each protein subunit can function to direct the ordered nucleation of inorganics or chemical cross-linkers for the display of novel molecules on either the inner or outer virion surfaces. Second, the ability of these particles to self-assemble and remain monodispersed in solution permits their functionalization and analysis in bulk suspensions. In fact, studies have determined that virion particles such as *Paramecium bursaria chlorella virus* (PBCV-1) behave similar to nanosphere polymer colloids in suspension, allowing standard materials science approaches to be used for their analysis (Sirotkin et al., 2014). Third, the three-dimensional (3-D) structures for many of these viruses are known, permitting the design and targeting of specific moieties on or within the PVN as a means to add functionality. Fourth, genetic programming for the development of virions with directed structural and functional properties is possible, including the addition of unique amino acids, peptides and assembly profiles. Fifth, as biomaterials plant viruses can be produced at scale and in a renewable fashion. We are dependent upon biomaterials for many of the necessities and conveniences of everyday life. Most of these biomaterials including ethanol fuels, cotton and wood fibers are produced through modern agricultural practices and at a scale that makes these products extremely cost efficient. The ability to apply such large-scale cost effective practices to produce nanoscale materials represents an attractive method for industrial applications. Combined these traits have attracted a range of researchers with expertise ranging from agronomic production, virology, structural biology, colloid chemistry and microdevice fabrication to explore the potential application of these particles in materials and therapeutic applications.

Plant viruses commonly investigated for nano-materials applications

Several plant viruses have been extensively investigated as scaffolds for materials applications. These viruses share several common traits that include the lack of an envelope, established structural information, stability under a range of conditions and simple methods for virion/PVNs production and purification. In addition, infectious cDNA clones for these viruses allow for the use of genetic engineering approaches for the modification of PVN structure (Chapman et al., 1992; Dawson et al., 1986; Eggen et al., 1989; Liu and Lomonossoff, 2002; Xiong and Lommel, 1991). Characteristics of several highly studied plant viruses used in nanofabrication studies are outlined below.

Icosahedral scaffolds

One of the structurally simplest plant viruses currently being investigated for its application as a nanoparticle is *Red clover necrotic mosaic virus* (RCNMV). The RCNMV virion is assembled from 180 copies of an identical coat protein (CP) subunit to form an icosahedral particle with an outer diameter of 36 nm and an inner diameter of 17 nm. Crystallographic and cryoelectron microscopy studies indicate RCNMV shares significant structural similarities with a related member of the *Tombusviridae*, *Tomato bushy stunt virus* (Martin et al., 2010; Sherman et al., 2006). One RCNMV feature that has made it a particularly attractive nanoparticle carrier is the ability to modulate pore openings and closings within the particle via divalent metal cations (Sherman et al., 2006). Similar to RCNMV, *Cowpea chlorotic mottle virus* (CCMV), a member of the *Bromoviridae*, produces a 26 nm diameter icosahedral virion composed of a 180 identical CP subunits and also undergoes reversible pore conversions in response to pH changes, providing a strategy for the loading and unloading of target molecules (Klem et al., 2005; Speir et al., 1995b; Tama and Brooks, 2002).

Another icosahedral virus that has been developed as a nanoparticle scaffold is *Cowpea mosaic virus* (CPMV) (Sainsbury et al., 2010). The CPMV virion is 30 nm in diameter and composed of 60 copies each of two different CP subunits. The small subunit is a 23 kDa peptide and folds into a jellyroll β-sandwich while the large subunit is a 41 kDa peptide composed of two domains each folded into a jellyroll β-sandwich (Lin et al., 1999). Additionally, CPMV can be purified in large quantities from infected leaves, is stable at temperatures up to 60 °C and can withstand some organic solvents (Montague et al., 2011; Steinmetz et al., 2009; Wang et al., 2002).

Filamentous scaffolds

The rigid rod (18×300 nm) virion of *Tobacco mosaic virus* (TMV) is the most widely investigated rod-shaped plant virus nanoparticle. The TMV virion consists of about 2130 identical 17.5 kDa CP subunits stacked in a helix around a single strand of plus sense RNA, leaving a 4 nm diameter channel through the 300 nm long virion axis (Namba et al., 1989). TMV and related members of the *Virgaviridae* have been propagated and purified in kilogram quantities using large scale industrial bioprocessing systems, demonstrating the potential to manufacture these scaffolds at a commercial level (Turpen, 1999).

Another filamentous plant virus that has received attention for its application to nanotechnology is *Potato virus* X (PVX). The PVX virion is a flexuous rod 13×515 nm in size and assembled from multiple copies of a single 25 kDa capsid protein. A low-resolution structure for the PVX virion has been reported (Kendall et al., 2013). In addition, extensive surface modifications have been investigated for the display of peptides and ligands by genetic and conjugation systems (Lee et al., 2014).

Functionalizing PVN scaffolds

A key use of PVNs in nanotechnology is the scaffolding of materials into novel physical configurations on the inner and outer virion surfaces. Simple plant-derived PVN scaffolds confer a number of advantages in this process including nanoscale control over position and spacing of the scaffolded materials, increased material concentration and activity through encapsulation within a defined space and high aspect ratio features for enhanced surface area. Seminal studies by Douglas and Young and Shenton et al. were among the first to utilize these advantages for the deposition of inorganics including paratungstate and decavanadate as well as cadmium and lead sulfides within the icosahedral cage of CCMV or along the outer surface of TMV, respectively (Douglas and Young, 1998, 1999; Shenton et al., 1999). These initial studies laid the groundwork for the development of protein structural modifications and novel chemistries that now permit the integration of mineralized PVNs into a range of applications. Outlined below are several examples of the strategies for the attachment and deposition of PVN templated materials.

Chemical conjugation

The protein shells of viral particles contain an array of amino acid side chains (e.g. lysine, cysteine, tyrosine, histidine) that provide reactive sites for the chemical conjugation of desired agents which include fluorescent and medical imaging dyes, protein or small molecule therapeutics and reactive peptides for sensor or cell entry (Franzen and Lommel, 2009; Lee et al., 2014; Li and Wang, 2014). Because of limited space we will not attempt to categorize the numerous chemical conjugation methods that have been used to functionalize viral capsids and instead direct the reader to several excellent reviews that cover this topic as well as provide strategies for selecting appropriate chemical conjugation schemes (Dedeo et al., 2011; Stephanopoulos and Francis, 2011; Strable and Finn, 2009).

Genetic engineering of new residues has also been used to position the conjugation site at the desired location either on the internal or external surfaces. For example, this approach has been utilized to produce 3-D arrays of Au nanoparticles (1.4–5 nm) on the surface of CPMV (Blum et al., 2004; Wang et al., 2002). By adding unique cysteine residues to the surface of CPMV Blum et al. was able to attach Au nanoparticles at defined spatial locations (Blum et al., 2005). CPMV scaffolded Au particles were then interconnected via thiol conjugations to produce conductive nanowires and blocks. The ability to use the uniform and repeating patterns of PVN reactive sites to design and control the contacts between 3-D arrayed PVNs is a novel approach to fabricate electrical circuitry at the nanoscale.

Electroless deposition

Electroless deposition (ELD) is a key method that has been extensively studied for the mineralization of biological templates with inorganic materials (Bittner, 2009). ELD reactions are ideally suited for biological templates as they are solution based, active at room temperature and pH neutral. In addition, ELD systems provide nanometer scale tunability for the density and thickness of the deposited coatings. This tunability is dependent upon the controlled and uniform reduction of metallic and oxide metals onto solution exposed PVN surfaces. The repeating patterns of charged amino acids along PVN surfaces provide molecular level

spacing from which to attract precursor ions such as Pd and Pt (Manocchi et al., 2011; Shenton et al., 1999). Typically the solution pH is adjusted so that the charges of the PVN surface and coating ions are mutually attractive (Knez et al., 2004; Lee et al., 2006). Subsequent reduction of scaffold associated ions produces the autocatalytic deposition of additional metals, forming nanoparticles that rapidly grow and coalesce to cover the entire PVN surface within seconds (Manocchi et al., 2011). Alterations to the viral template that enhance the attraction between the virus scaffold and precursor ion, such as the genetic engineering of surface exposed cysteine derived thiol groups, can significantly enhance the uniformity and tunability of this coating process (Blum et al., 2005; Lee et al., 2006; Royston et al., 2008). Additional ELD modifications including the addition of PEG-thiol groups or repeated deposition cycles have also been demonstrated to enhance metal deposition onto viral templates (Lim et al., 2010; Zhou et al., 2012).

Polymer and silica coatings for added stability and function

PVNs have been functionalized with silica and polymeric coatings such as aniline which are both stable in a wide variety of chemical and physical conditions. These polymer coatings are known to enhance the stability of virus scaffolds under more stringent coating processes as well as yield novel materials with unique conductive and mesoscale structures (Fowler et al., 2001; Niu et al., 2007a, 2007b; Rong et al., 2009). The uniform size, shape and molecular properties (e.g. charge, hydrophobicity) of PVNs generally function to drive particle-to-particle associations into higher order arrays and films in a concentration dependent manner (Rong et al., 2011). These self-association properties have been applied to the production of 2-D films and monolayers with potential applications in nanoelectronics and sensing. One example of functionalizing PVNs to form discrete macromolecular complexes involves the end-to-end alignment of rod-shaped TMV. The polar nature of the TMV rod results in a natural propensity to align end-to-end (Butler, 1999). However, this end-to-end association is concentration dependent and unstable. Niu et al. addressed this instability by polymerizing a nanometer thin layer of polyaniline onto the TMV PVNs (Niu et al., 2006, 2007b). This polymer coating neutralizes repulsive carboxylate interactions at the rod ends resulting in stable single virion width fibers greater than 1 μm in length (Lu et al., 1996; Niu et al., 2006).

Aniline coated PVNs can be further modified by silica condensation reactions to produce thick (50–100 nm) silica shells that are amenable to a number of modifications under conditions that would normally denature the virus by itself (Royston et al., 2009). These silica encased virus templates provided a highly stable and robust platform for the deposition of metals at high densities and applications that require organic solvents and high temperatures including catalysis and molecular separations.

Genetic engineering of extended CP sequences

Genetic modifications to CP open reading frames (ORFs) have also provided a successful strategy to produce novel PVNs with unique functionalities. In general the addition of short peptide sequences, ~15 to 25 amino acids, are tolerated for virion assembly (Pogue et al., 2002). However, assembly appears to be highly dependent on the nature of the peptide sequence such that even short extensions can inhibit virion formation (Frolova et al., 2010). Larger peptide additions to the virion surface including fluorescent proteins GFP and mCherry have been reported for PVX (Cruz et al., 1996; Tilsner et al., 2013). The assembly of these larger CP fusions depends on the co-expression of the wild-type CP either independently or through the use of a “leaky” stop codon, producing a particle with a mix of CPs of which only a fraction displays the fusion peptide (Cruz et al., 1996). In contrast, Werner et al. demonstrated that fusion of a 133 amino acid segment of protein A to the TMV CP ORF produced virions that displayed this peptide from every subunit, conferring antibody binding to the entire virus particle (Werner et al., 2006). This finding further demonstrates the importance of the extension sequence in achieving assembly of the modified CP. From these studies it seems clear that virus CPs are remarkably pliable for their ability to assemble and thus amenable to a range of modifications.

Templating novel materials and functions

PVN nanowires

The 4 nm central channel of the rod-shaped TMV particle has been used as open-ended container from which to confine the deposition of a range of metals and metal alloys including Ag, Ni, Co, Cu, Pt, Co – Pt, and Fe – Pt (Balci et al., 2006; Dujardin et al., 2003; Knez et al., 2003; Kobayashi et al., 2010). Selective deposition within the TMV PVN inner channel is achieved by tuning the activation of Pd and Pt precursor ions with phosphate buffer such that in the absence of phosphate ions ELD occurs primarily within the inner channel while in the presence of phosphate buffers deposition occurs on the outer surface (Knez et al., 2004). Constrained within the inner channel these deposition reactions produced 3 nm wide wires of varying lengths. Genetic modifications to the inner channel of *Tomato mosaic virus* (ToMV), a close relative to TMV, have been applied to enhance and alter the synthesis of metal wires within the PVN inner channel (Kobayashi et al., 2010). Amino acid substitutions along the inner face of the ToMV inner channel were created to increase the number of positively charged nucleation sites for the attraction of precursor cations resulting in enhanced inner channel coatings with the alloy Co – Pt. These hybrid metal-PVN particles have potential applications in catalysis and sensing. It is interesting to speculate that PVN CP insulated conductive wires could be further decorated with binding peptides to produce sensor architectures that position the analyte binding site within a few angstroms of the electrode sensing surface. Such sensor architecture could significantly enhance signaling at the biology-device interface.

Scaffolds and cages

The interior surfaces of virus particles represent constrained cargo containers. Evolution has designed these containers to preferentially accept viral nucleic acid through a process orchestrated by sets of electrostatic interactions between basic amino acids, arginine and lysine within the viral CP nucleic acid binding domain and the negatively charged viral genome. The positive charges within the binding domain represent a useful surface from which to attach and synthesize cationic compounds. Douglas et al. (2002, 1998) demonstrated that the positively charged interior surface of empty (eCCMV) particles provides a constrained reaction vessel for the synthesis of anionic materials including Fe_2O_3, vanadates, tungstates and molybdates (Fig. 1A and B). Similarly, genetic mutations that replaced nine basic residues at the internal N-terminal domain of the CCMV CP with negatively charged glutamic acid produced a conducive nanometer-scale cage for the synthesis of cationic transition metals Fe_2O_3, Fe_3O_4, Co_2O_3 (Douglas et al., 2002). Synthesis within the eCCMV particles was controlled by reversible pH-regulated gating that induces the opening of virion pores upon treatment at pH > 6.5 (Speir et al., 1995a). Similar capsid gating is observed in RCNMV where it has been used to load and release small molecules including

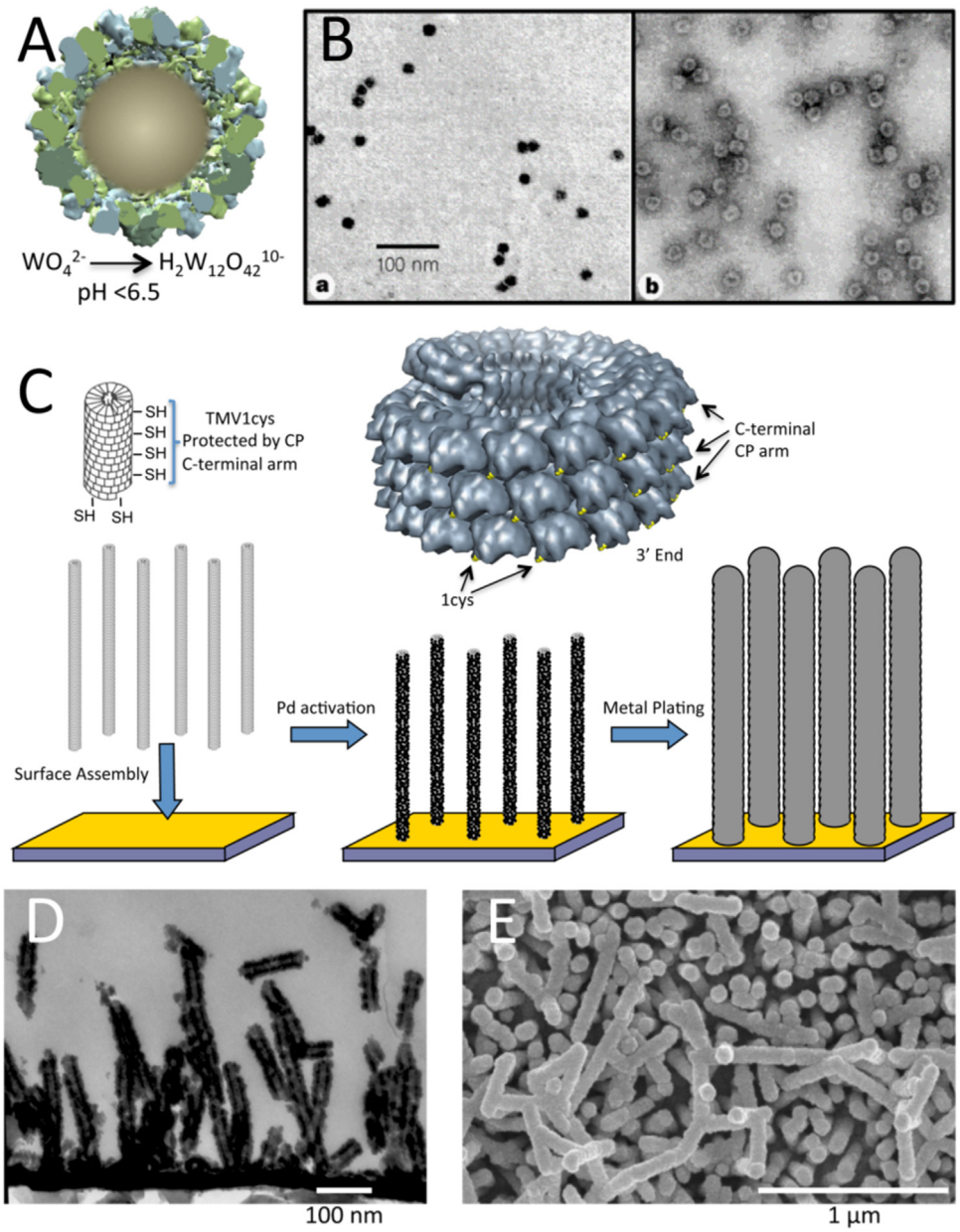

Fig. 1. PVN mineralization and surface assembly. (A) Tungstate ions enter open eCCMV at pH > 6.5. Particle closure and mineralization occur upon lowering pH. (B) unstained and stained TEM images of paratungstate mineralized eCCMV. Reprinted with permission from T, Douglas, and M. Young, Nature 393, 152 (1998). Copyright 1998, Nature Publishing B, TMV1cys assembly and mineralization via electroless plating. C and D, TEM and SEM images of nickel coated TMV 1cys assembled surfaces. Adapted with permission from E. Royston, A. Ghosh, P. Kofinas, M.T. Harris, and J.N. Culver, Langmuir 24(3), 906 (2008). Copyright 2008. American Chemical Society.

fluorescent dyes and cancer therapeutics (Honarbakhsh et al., 2013; Loo et al., 2008).

Inside/outside PVN modifications for therapeutic applications

The ability to deposit and cage molecules within a nanoparticle is a highly sought trait for applications in therapeutic and imaging dye delivery (Franzen and Lommel, 2009; Wen et al., 2013). Much of this interest is derived from the ability to precisely functionalize defined PVN surfaces with tracking dyes, therapeutic payloads and cell specific ligands. We will not attempt to fully address this rapidly expanding area of study but instead highlight a few recent studies demonstrating the potential of these systems.

RCNMV PVNs have been studied for the uptake and release of the cancer drug Doxorubicin (Dox) (Cao et al., 2014). The loading of Dox onto and within the RCNMV PVN comprises two distinct processes. The first involves electrostatic interactions between the drug and surface residues of the RCNMV particle. These surface-based electrostatic interactions can be modulated through environmental factors (buffer composition, pH, ionic strength) to control drug loading and release. The second involves loading of the drug within the PVN via its pore openings and appears to involve the intercalation of the flat ringed Dox molecules with the encapsidated viral RNA. Dox release from within the PVN was found to be slower presumably due to stronger binding affinities with the viral RNA.

In another study the ability to precisely modify TMV PVNs genetically and chemically at defined positions allows for the design of nanoparticles that carry multiple functions for both imaging and cell targeting. Bruckman et al. has utilized this multimodality approach to attach fluorescent dyes for optical imaging, Gd ions for magnetic resonance imaging and a receptor peptide to

target cell adhesion through the VCAM-1 receptor (Bruckman et al., 2014). These multi-functional high aspect ratio rod-shaped TMV PVNs were found to enhance the detection of atherosclerotic plaques within blood vessels of a mouse model. It is clear from these studies that PVNs hold promise as cargo carriers for a range of medically relevant materials.

PVN assembly and patterning for enhanced applications

One of the most significant challenges in developing biological templates for applications in nanoscience is the ability to precisely pattern and integrate these templates into devices so as to leverage their nanometer size and enhance function. Several approaches that have permitted the integration and fabrication of functionalized plant PVNs onto surfaces for enhanced applications are described below.

Two-dimensional surface assemblies

Surface alignments of both icosahedral and filamentous plant-based PVNs have been accomplished using evaporative methods such as drop-and-dry or solution dipping. Formation of PVN films by these methods is predominantly controlled by the concentration of PVN and the strength of its surface attraction (Barick and Bahadur, 2010; Evans, 2008). The uniform and multivalent nature of these PVN coated surfaces has led to investigation into their effects on cell responses and differentiation (Kaur et al., 2010a, 2010b; Zan et al., 2012; Zeng et al., 2011). Using *Turnip yellow mosaic virus* (TYMV) as a scaffold for the multivalent display of the cell adhesion peptide RGD, Zan et al. demonstrated enhanced mesenchymal stem cell adhesion and spreading onto surfaces layered with TYMV genetically programed to display the RGD peptide (Zan et al., 2012). Similarly, surfaces coated with TMV particles have been shown to facilitate the differentiation of mesenchymal stem cells into bone producing osteogenic cells. In the presence of TMV coated surfaces, stem cells showed a marked induction of bone morphogenetic protein-2 and coalesce to form bone-like nodules within 24 h (Sitasuwan et al., 2012). In a subsequent study differentiation into osteogenic cells was also enhanced by surface featured TMV PVNs chemically cross-linked with the RGD adhesion peptide (Sitasuwan et al., 2014). Combined these studies demonstrate that plant-based PVNs provide multivalent display scaffolds for the display of nanotopographical features capable of promoting cell adhesion and differentiation.

Another application for the use of surface displayed metal nanoparticles is in catalysts where there exists a range of industrial reactions in which the unique material properties and increased surface area of nano-feature catalysts can confer enhanced activities over similar bulk displayed materials (Zhang et al., 2014). In addition, the ability to modulate catalyst position, size and spatial density represents a significant means to control catalytic reactions. PVNs have provided a unique backbone from which to produce, anchor and display key nano-catalysts. As an example, Yang et al. utilized the TMV PVN to controllably produce and display Pd nanoparticles of defined size, 5–15 nm in diameter, and distribution (Yang et al., 2014). Reactions directed at the catalysis of hexavalent chromium, a toxic environmental pollutant, using these TMV templated Pd nanocatalysts produced significantly higher catalytic activity per unit Pd mass than commercial Pd – carbon systems.

3-D assemblies

Icosahedral viruses represent highly uniform 3-D building blocks that are amenable for the assembly of multi-dimensional nano and meso scale structures. For example, CPMV and CCMV have both been investigated for their ability to form layered 3-D surface structures using biotin - streptavidin crosslinking (Steinmetz et al., 2006; Suci et al., 2006). This approach allows the layer-by-layer assembly of functionally unique PVNs to produce micron level ordered particle assemblies. Kostiainen et al. used tunable electrostatic attractions between negatively charged CCMV particles and positively charged gold nanoparticles to produce 3-D superlattices (Kostiainen et al., 2013). Such layer-by-layer assembly processes combined with the ability to modify both outer and inner PVN surfaces represent a powerful method for the precision assembly of multifunctional nanoscale materials.

Filamentous plant viruses represent high aspect structures with the potential to significantly increase surface area if they could be patterned in a vertical manner. To address this potential the rigid rod of TMV was genetically engineered to promote vertical alignment and surface attachment (Royston et al., 2008) (Fig. 1C – E). Using the known 3-D structure of TMV a novel mutant, TMV1cys, was created by inserting a cysteine codon within the N-terminus of the CP ORF (Namba et al., 1989). Through thiol-metal or thiol-charge interactions the positioning of the 1cys mutation contributes to the attachment

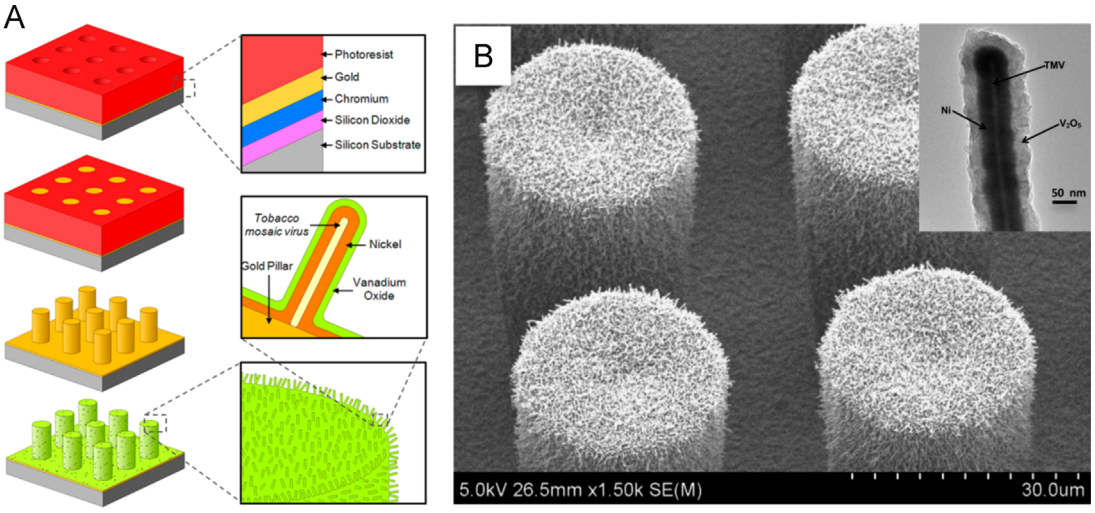

Fig. 2. PVN based hierarchical surface fabrication. (A) Fabrication schematic. (B) SEM image of surface pillars assembled with TMV-PVNs. TEM inset image of a single TMV1cys pillar. Reprinted with permission from K. Gerasopoulos, E. Pomerantseva, M. McCarthy, A. Brown, C. Wang, J.N. Culver, and R. Ghodssi, ACS Nano 6(7), 6422 (2012). Copyright 2012, American Chemical Society.

and vertical positioning of the viral rods onto a variety of surfaces including gold, stainless steel, SU-8 polymer and Teflon™ (Gerasopoulos et al., 2010; Ghosh et al., 2012; Royston et al., 2008). Although surface exposed, the N-terminal 1cys mutation is recessed within a groove and partially covered by the C-terminal arm of the CP. This position likely inhibits direct contact between the cysteine derived thiol and the metal surface except at the 3′ end of the virion rod where the thiol group is sufficiently exposed to make direct surface contact. Subsequent ELD mineralization of surface assembled TMV1cys produce evenly coated virus templates containing confluent metal coatings as thin as 15 nm in thickness. In addition, the virus rods are perpendicularly assembled onto surfaces, significantly increasing available area by an order of magnitude (Royston et al., 2008). Surface assembly of viral particles prior to inorganic coatings also represents a means to overcome issues associated with the aberrant metallization of non-templated particles (Knez et al., 2004; Lee et al., 2005; Shenton et al., 1999). Viral template attachment to a surface allows for these non-templated clusters to be washed away. This process provides a simple and robust means to produce nanofeatured surfaces with increased area that can be directly integrated onto device interfaces.

Top-down methods for PVN fabrication

The ability to integrate biology into functioning devices has revolutionized genomic and proteomic analysis and their use in sophisticated surveillance systems for the detection of disease and defense threats (Liu et al., 2010). The inherent stability of PVNs makes them compatible with several traditional top−down microfabrication methods. For example, Gerasopoulos et al. utilized a lithography-based lift-off method to pattern TMV1cys assembled on gold-coated silicon wafers (Gerasopoulos et al., 2010). This method utilizes photoresist masks to define sacrificial layers that are removed by either acetone for metal-coated PVN surfaces or a pH adjusted developer for naked uncoated PVNs to reveal the desired surface patterns.

Plant-based PVNs have also been patterned into nanostructures using electron beam lithography (eBL) (Alonso et al., 2013b). In this study TMV PVNs were spin coated with either negative or positive resist and eBL writing used to expose defined PVN sections while leaving other particle sections covered. The modified system developed for this process utilizes biocompatible temperatures and organic solvents resulting in a structurally intact TMV particle that was readily decorated with CP specific antibodies.

It is also possible to enhance the stability of TMV in organic solvents and at temperatures up to 160 °C. Holder et al. accomplished this increase in stability though the conjugation of polyethylene glycol chains onto surface exposed CP tyrosine residues (Holder et al., 2010). The protective PEG coat allows the TMV PVN to be integrated with hydrophobic materials and organic solvents while maintaining the ability to further modify additional CP residues. As a result PEG modified viruses can be thermally cast within solid polystyrene, opening up the potential to incorporate functionalized virus scaffolds into conductive polymers for enhance photoelectronic and sensing applications.

PVNs have also been shown to be suitable for patterning by contact printing methods. Specifically, a polydimethylsiloxane stamp was used to create oriented lines of TMV PVNs onto oxidized silicon surfaces with feature spacings as small as 130 nm (Balci et al., 2008). The ability to treat PVNs as ink opens an array of technologies for the large scale patterning of functionalized PVNs onto device surfaces. Combined, these top-down approaches provide conceptual advances toward the top-down fabrication of complex biological material into device applications. The ability to pattern naked PVN templates via these microfabrication methods without disrupting their structure or activity demonstrate the potential of these nanotemplates to be integrated into traditional manufacturing streams.

Applications of surface assembled PVNs

PVN-based electrode enhancements

The nanostructured features produced by the surface assembly of TMV1cys confer a number of advantages to electrodes over planar surfaces. Most significantly the high aspect ratio structure of the TMV rod results in a significant increase in surface area, permitting greater contact between electrode and electrolyte. Virus nanostructured surfaces also confer unique electrode architecture where metal-coated TMV1cys viruses function as an array of current collectors, each one surrounded by energy active material (Fig. 2). To produce these electrodes TMV1cys is first assembled onto a current collector such as stainless steel and then coated by ELD with conductive nickel or cobalt. Several fabrication methods including atomic layer deposition, sputtering, electrodeposition or polymer electrolyte deposition are then used to deposit specific battery active materials over the virus-featured surfaces (Chen et al., 2011a, 2010, 2011b, 2012; Gerasopoulos et al., 2010, 2012; Ghosh et al., 2012; Pomerantseva et al., 2012) (Fig. 2). This architecture greatly reduces the diffusion lengths for both electrons and ions during battery cycling, producing greater energy storage at a given discharge rate and faster overall charge discharge capabilities.

TMV1cys has been investigated as an electrode material in a number of battery chemistries including nickel-zinc and lithium-ion (Chen et al., 2011a, 2010, 2011b, 2012; Gerasopoulos et al., 2008; Royston et al., 2008). Here we will only highlight one TMV-based electrode application involving the integration of TMV1cys-featured surfaces for the construction of silicon-based anodes (Chen et al., 2011a, 2010). Silicon has the highest potential energy density for any anode material (Boukamp et al., 1981). However, upon lithium cycling silicon undergoes considerable swelling, leading to pulverization of the silicon coating and subsequent poor cycling behavior. Efforts to identify electrode architectures that tolerate this swelling have identified columnar nanowire structures as structurally accommodating (Chan et al., 2008). The rod-shaped TMV1cys represents a novel columnar nanowire structure that when coated with conductive nickel provides a forest of nanoscale electrodes that can be coated with silicon by vapor deposition or electrodeposition (Chen et al., 2011a, 2010). These virus-based silicon composite electrodes exhibited significant improvements in cycling stability as well as electrochemical activity due to the unique core-shell proximity between the conductive nickel within each silicon nano-column. Furthermore, PVN-featured silicon anodes produced energy densities that were nearly three times that of commercially used graphite (Chen et al., 2011a).

In addition to providing a nanostructured scaffold for the fabrication of nanocomposite electrode materials surface attached TMV1cys also stabilizes the association of the electrode material on the fabrication surfaces. Ghosh et al. assembled TMV1cys on to a non-stick Teflon™ surface (Ghosh et al., 2012). Subsequent coatings with cobalt oxide and a flexible polymer electrolyte were possible only on TMV1cys and not with the unmodified wild-type TMV. This indicates that surface assembly is dependent upon interactions between the electronegative Teflon™ surface and the TMV1cys engineered cysteine-based thiol groups. Intercalation into the 3-D TMV1cys surface and polymerization of a polymer electrolyte produced a peelable electrode. Thus, in addition to enhanced surface area PVN nanofeatures also enhance the stability of ELD surface coatings.

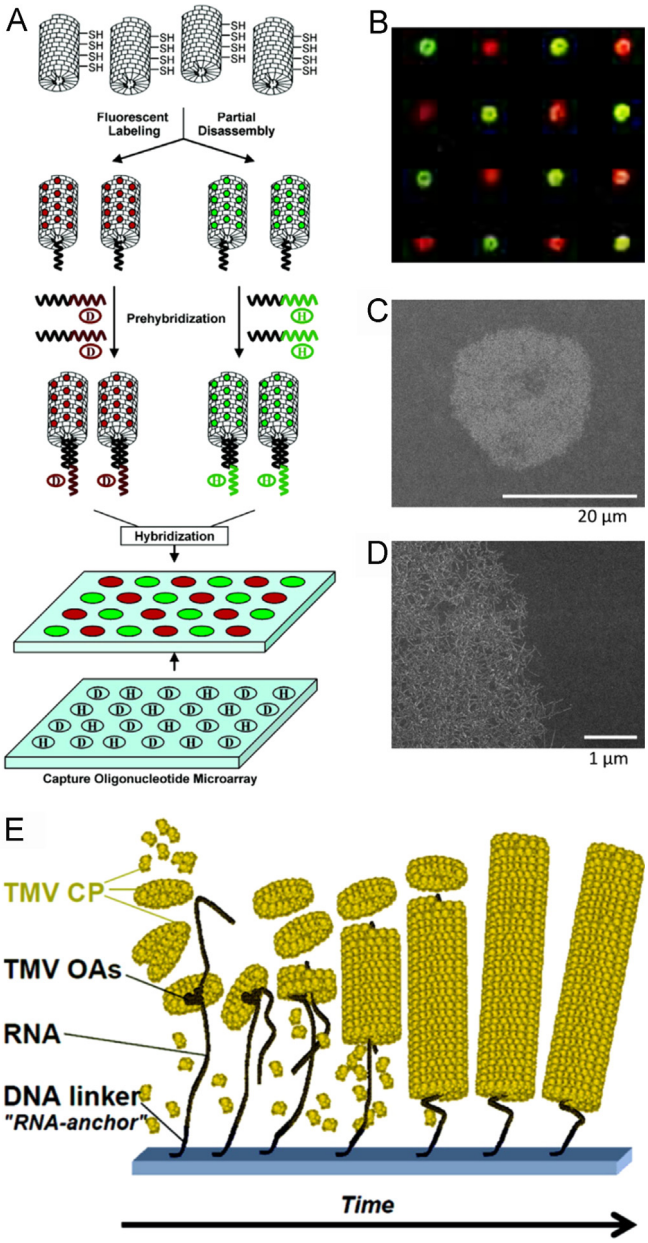

Fig. 3. Nucleic acid directed patterning and assembly of TMV PVNs. (A) Scheme for the patterned hybridization of partially disassembled fluorescently labeled TMV PVNs. (B) (C) and (D) Fluorescent micrograph and SEM images of patterned TMV PVNs. Adapted with permission from H. Yi, G.W. Rubloff, and J.N. Culver E, Langmuir, 23(5), 2663 (2007). Copyright 2007, American Chemical Society. E, Diagram for the assembly of surface attached TMV RNA with CP, dimensions not to scale. Reprinted with permission from A. Mueller, F.J. Eber, C. Azucena, A. Petershans, A.M. Bittner, H. Gliemann, H. Jeske, and C. Wege, ACS Nano 5(6), 4512 (2011). Copyright 2011, American Chemical Society.

Superhydrophobic surfaces, heat exchangers and photoelectrochemical cells

There have been a number of applications that combine the bottom-up assembly of virus nanofeatures with top – down nanofabrication methods. One such application is the fabrication of hierarchical structures that combine both nanoscale and microscale structures to produce superhydrophobic surfaces. McCarthy et al. used a biomimetic approach to reproduce the hierarchical features responsible for the hydrophobicity of aquatic plant leaves (McCarthy et al., 2012). Using traditional lithography methods microscale SU-8 polymer columns were created and assembled with TMV1cys PVNs as a mimic of the microscale bumps and nanoscale hairs found on lotus leaves. Results demonstrated that surfaces with PVN based nano-features produced an antiwetting capillary environment. When combined with the ability of the micro-scale columns to disrupt water droplet shockwaves, a unique non-wetting substrate was produced that closely mimics that of plants and represents a paradigm shift in the design of water repellent surfaces.

Heat exchange surfaces represent another application where TMV1cys-featured surfaces confer substantial increase in efficiency. Nickel plated TMV1cys gold, copper, aluminum and stainless steel surfaces show increased heat transfer coefficients up to 200% while showing no physical degradation of the PVN coated surface after a 24 h boiling exposure (Rahman et al., 2014). Heat transfer represents an important aspect in many industrial applications such as water purification and energy generation. Plant-produced PVNs thus represent a potentially cost effective means of enhancing this process on a large scale.

An additional application demonstrated for 3-D assembled TMV1cys has been in the production of nanofeatured surfaces for the enhancement of photoelectrochemical solar cells for the splitting of water to produce hydrogen gas (Chiang et al., 2012). In this study TMV1cys was assembled onto a gold-coated ITO/glass substrate, plated with nickel using ELD and sputter-coated with photoreactive CuO to a thicknesses of 500 nm. This PVN nanoscale configuration was designed to reduce the charge carrier transport distance. Because the resulting nanostructures are smaller than the wavelength of visible light, light reflection is decreased and more solar energy is absorbed. Results from this configuration produced the highest yet recorded photocurrent density; 3.15 mA/cm^2 for similar sized CuO systems.

Nucleic acid modifications for PVN patterning and assembly

The development of “DNA origami” methods that utilize nucleic acid hybridization to produce novel macromolecules of defined shape and size have transformed our expectations regarding the structural control that is achievable with biological components (Rothemund, 2006). To date several approaches have been investigated as a means to incorporate the specificity of nucleic acid hybridization into the patterning or modification of plant-based PVNs. One study has taken advantage of the natural disassembly steps of TMV to add specific DNA linkages that can be used to address the virus particles to defined locations that contain the complementary DNA sequences (Yi et al., 2005, 2007) (Fig. 3A – D). TMV disassembles via a co-translational process in which the increased pH and reduced Ca^{++} concentrations that occur upon cell entry destabilize juxtaposed intersubunit carboxylate groups (Namba et al., 1989; Wilson, 1984). This weakens the virus particle and leads to the preferential removal of CP subunits from the viral RNA 5′ end where the protein-nucleic acid interactions are weakest due to the lack of guanosine residues within the first 69 nucleotides of the TMV genome (Steckert and Schuster, 1982). During infection this produces a rod-shaped particle with an exposed ribosome-binding site, allowing the virus RNA to remain encapsidated until under active translation. This process can be mimicked in vitro to produce PVNs with exposed 5′ RNA sequences. Yi et al. took advantage of this natural process to hybridize unique DNA linker sequences to the viral 5′ RNA sequences (Yi et al., 2005, 2007). These linker sequences also encoded novel address sequences that enabled hybridization patterning of the PVNs to surface printed complementary sequences. This system has subsequently been used to pattern TMV displayed antibodies onto discrete hydrogel microparticles, producing a 53-fold enhancement in target protein capture from

the displayed antibodies (Jung and Yi, 2014). This level of protein capture represents a significant enhancement over traditional systems and has the potential to incorporate nucleic acid directed PVN based antibody patterning to isolate and sort target proteins from complex solutions.

Viral nucleic acids encode origin of assembly (OAS) sequences that function in association with the CP to initiate virion assembly (Basnayake et al., 2009; Butler, 1999). In addition, OAS sequences are modular and when engineered into a heterologous nucleic acid sequence can direct the encapsidation of that sequence (Hwang et al., 1994). Interestingly, Loo et al. attached an oligonucleotide mimic of the RCNMV OAS to Au, CdSe and $CoFe_2O_4$ nanoparticles, 3–15 nm in diameter (Loo et al., 2007). The addition of RCNMV CP and RNA 1 resulted in the assembly of virus-like capsids around the targeted nanoparticle, demonstrating that OAS sequences can function to direct the encapsulation of inorganic materials.

Viral OAS sequences have also been used in the assembly and patterning of TMV PVNs using RNAs covalently ligated to DNA linkers conjugated onto pre-patterned aldehyde-functionalized SiO_2 wafers (Mueller et al., 2011) (Fig. 3E). Patterned RNAs containing the OAS were capable of guiding the bottom-up assembly of TMV-like rods, 100–500 nm in length, upon the addition of purified CP. The ability to assemble a TMV-like rod on an isolated surface attached RNA holds the potential to produce chimeric capsids that display unique functional groups or peptides at precise locations along the length of the PVN. Recently, the production of RNAs that contain multiple TMV OAS in defined positions have been used to produce branched rod-shaped particles (Eber et al., 2015). This approach represents a potential strategy to incorporate nucleic acid origami design principles into the direct assembly of PVNs. In addition, the ability to surface immobilize virion assembly intermediates on defined RNA templates represents a potentially powerful means to capture and directly identify CP intermediates that are involved in virion initiation and elongation. Such a system could address the longstanding controversy as to the structural configuration of the CP aggregates involved in the assembly process (Butler, 1999; Correia et al., 1985).

Empty plant virus nanoparticles (ePVNs): strategies and applications

PVNs have proven to be both genetically and chemically malleable to the addition of novel moieties that add new functionalities to the virus nano-structure (Flynn et al., 2003; Liu et al., 2012; Pokorski and Steinmetz, 2011; Saunders and Lomonossoff, 2013). Yet despite these characteristics the application of intact virus particles is often limited by problems associated with viral replication and recombination that lead to the deletion of the desired function. In addition, genome size constraints dictated by the need for a replication-competent virus genome also limits the types of genetic modifications that can be made to these particles. To circumvent these limitations researchers have for a number of years developed heterologous expression systems for the production of virus-like particles or ePVNs that assemble in the absence of the viral genome (Dedeo et al., 2011; Saunders and Lomonossoff, 2013; Yildiz et al., 2011). ePVNs eliminate the need for infection competent virus while expanding the genetic programmability of the PVNs and decreasing time of production. Described below are selected examples of ePVN systems and applications.

Strategies for ePVN production and controlled assembly

There are well-established examples of icosahedral virus CPs self-assembling into empty particles when expressed from a heterologous system such as *Escherichia coli* and *Pichia pastoris* (Brumfield et al., 2004; Dedeo et al., 2011; Sainsbury et al., 2010). While we will not describe these established heterologous expression systems in detail we do want to highlight the development of a CPMV system for the generation of empty VLPs directly in plants (Saunders et al., 2009). To achieve ePVN assembly in plants Saunders et al. generated a set of high expression vectors that take advantage of a modified leader sequence from CPMV to induce hypertranslation of cognate mRNAs (Sainsbury and Lomonossoff, 2008). Using this system a precursor VP60 ORF was co-expressed with the viral 24 kDa proteinase to yield mature L and S CPs that are assembly competent. This plant-based system for the production of ePVNs provides potential advantages in the scale of production as well as cost savings.

The ability to express and assemble PVNs independent of virus infection also holds the potential to produce novel particle architectures that would not normally be created. For example, the assembly profile of CCMV-derived ePVNs can be modulated between icosahedral and rod-like by encapsulation of a polyanionic semiconducting polymer (Ng et al., 2011). The polyanionic polymer functions as a stand-in for the negatively charged viral RNA, promoting PVN assembly from purified CCMV CPs. Solution ionic strength is used to modulate the structure of the polymer from a coiled form, which results in icosahedral PVNs similar to the virus, to an extended conformation, which produces rod-like CCMV structures. This process appears to mimic that of a number of multipartite plant viruses, such as *Alfalfa mosaic virus*, that produce a defined range of virion structures from icosahedral to rod-like depending on the length of the encapsidated nucleic acids (Kumar et al., 1997).

Strategies for the production of empty rod-shaped TMV ePVNs have also been developed (Brown et al., 2013). Generally, monomer and trimers of the TMV CP assemble into a two-layer disk composed of 34 subunits that further assemble into virus-like rods in the presence of viral RNA (Durham et al., 1971; Klug, 1999). Factors including CP concentration, pH and ionic conditions can be used to control the equilibrium between these structural intermediates, even in the absence of viral RNA. The ability to control and modify the formation of assembly intermediates represents a powerful means to produce novel ePVN structures that would not form under native conditions. Previous studies have expressed TMV CP and shown that under physiological conditions (neutral pH) these purified CPs form only lower order assemblies that include small aggregates and disks (Bruckman et al., 2011; Dedeo et al., 2010, 2011; Miller et al., 2007). Recently, a bacterial optimized TMV CP ORF was modified by substituting charge neutralizing amino acids Q and N at position E50 and D77, respectively (Brown et al., 2013). Both E50 and D77 form part of an intersubunit carboxylate pair that functions to control virion assembly (Lu et al., 1996, 1998). Neutralization of these negatively charged amino acids negates the repulsive intersubunit interactions and stabilizes the quaternary structure of the helical rod even in the absence of viral RNA. As a result of these modifications the TMV CP self-assembled into ePVN rods. In addition, these ePVNs can be easily purified from lysed *E. coli* extracts in large quantities via gradient centrifugation. Subsequent studies have shown that the purified ePVNs contain no RNA and show a level of stability that is similar to the wild-type virus. These ePVNs can also be modified with the 1cys mutation and self-assembled onto device surfaces in a vertical fashion, functioning as biotemplates for the ELD plating of various metals. Furthermore, this system allows more extensive modification of the viral CP to display novel functionalities such as the surface display of peptides that would not otherwise be tolerated during virus infection. A range of expression constructs permit the display of defined peptides on all of the assembled subunits via direct fusion to the CP ORF or on only ~25% of the CP subunits through an amber stop codon (Brown et al., 2013).

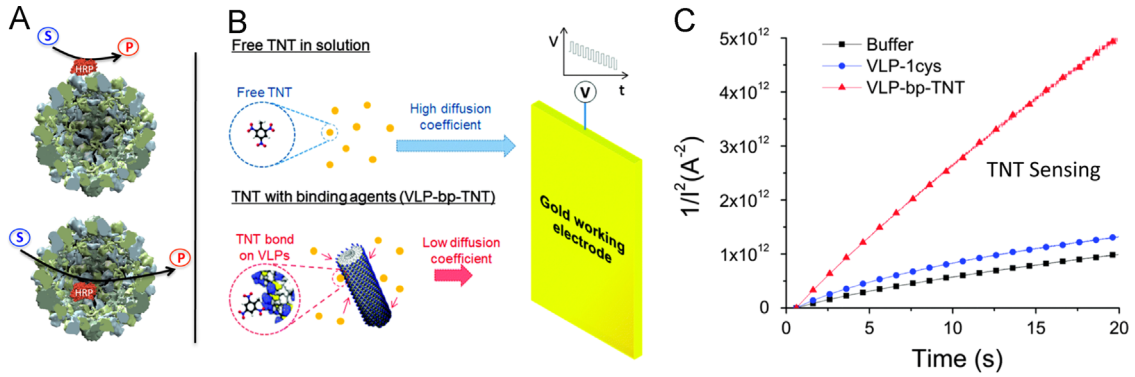

Fig. 4. PVN nanoreactors and TNT sensors. (A) Models for enzyme conjugation on external or internal CCMV surfaces, modified from Comellas-Aragones et al., 2007. (B) Diagram of TMV-VLP displayed TNT binding peptides (VLP-bp-TNT) for electrochemical sensing. C, Results of TNT sensing showing detectable peak changes for VLP-bp-TNT in the presence of TNT vs buffer and control constructs. Reprinted with permission from F. Zang, K. Gerasopoulos, X.Z. Fan, A.D. Brown, J.N. Culver and R. Ghodssi. Chem. Commun. 50, 12977 (2014). Copyright 2014, The Royal Society of Chemistry.

Additional TMV CP modifications have been produced as a means to promote and control the formation of TMV ePVN rods. Bruckman et al. genetically inserted a hexa-histidine tag on the C-terminus of the TMV CP (Bruckman et al., 2011). Bacterial expressed and purified CPs showed altered assembly profiles, forming hexagonal disk arrays and rafts of elongated rods whose assembly could be controlled by the concentration of nickel-nitrilotriacetic acid. In a subsequent study a novel cysteine residue introduced at position 103 in the inner loop of the TMV CP was found to drive virus-like rod assembly by disulfide formation between apposing CP subunits (Zhou et al., 2013). Redox driven disulfide bond formation between the 103 subunits was sufficient to stabilize CP assembly into virion-like rods. These findings demonstrate novel strategies through which external stimuli such as nickel ions or redox activity could be used to control TMV ePVN assembly and disassembly.

Dedeo et al. (2010) took alterations in the TMV PVN surface features to their extreme, utilizing protein design strategies to reposition the N and C terminus of the TMV CP from the outer PVN surface to the inner channel surface. This circular permutant CP was expressed from *E. coli* and shown to assemble into disks and TMV-like rods upon low pH treatments. Furthermore, the repositioning of the N and C terminus to the inner channel permitted the application of novel conjugation chemistries to the N terminus for the selective attachment of target molecules within the inner channel of the TMV-VLP rod (Schlick et al., 2005). These combined studies demonstrate the flexibility of virus assemblies to be designed and manipulated for the production and control of nanoscale assemblies. It seems likely that these studies represent only the beginning of ever more complex engineering designs that utilize PVNs to produce meso and microscale assembles from these macromolecular building blocks.

ePVN applications

Light harvesting and the study of charge transfer interactions

The symmetry of CP side chains within an assembled ePVN provides a means to spatially array light harvesting chromophores on a molecular scale that mimics the photosynthetic antenna complexes of plants. Several studies have used TMV CPs to assemble arrays of donor and acceptor chromophores from which energy transfer and light harvesting activity were readily measured (Endo et al., 2007; Miller et al., 2007, 2010). In these studies chromophores were cross-linked to engineered amino acids designed to position the aromatic chromophores within the inner channel or along the surface of assembled structures. Mixtures of CPs containing linked acceptor and donor chromophores were assembled into either disks or rods depending on pH conditions. Spectroscopy measurements revealed that under light activation energy transfer could occur from multiple donor chromophores to acceptors. Combined these studies demonstrate the flexibility of PVNs to function as models for understanding fundamental aspects of light harvesting.

Nanoreactors for enzymatic and therapeutic conversion

Within cells, most enzymes and enzymatic pathways are generally spatially confined as a means to control and enhance their activity. A number of studies have shown that enzyme encapsulation within ePVNs is a viable biomimetic approach to reproduce this cellular architecture and improve targeted enzymatic functions. One of the first to investigate ePVNs as enzymatic reaction vessels was Comellas-Aragones et al. who assembled the 44 kDa horseradish peroxidase enzyme (HRP) within CCMV CP (Comellas-Aragones et al., 2007) (Fig. 4A). Using the pH driven self-assembly of CCMV CP an assembly ratio of CP dimers to HRP was selected to produce ePVNs containing only one HRP molecule. Studies confirmed the functional activity of the encapsulated HRP and the ability to control this activity by regulating substrate access through the pH controlled gating of the CCMV pores. These findings outline a strategy to control ePVN based nanoreactors via access to the encapsulated enzyme. Similarly, Minten et al. has encapsulated both *Pseudozyma antarctica* lipase B (PalB) and EGFP within a single CCMV ePVN (Minten et al., 2011). Results indicated that the encapsulated PalB produced a higher activity than non-encapsulated enzyme and that only one PalB molecule per ePVN was required to achieve maximum activity. The ability to assemble CCMV ePVNs with defined ratios of PalB and EGFP also demonstrates the potential to encapsulate more complex enzymatic pathways to produce efficient nanoscale reactors.

Many cancer therapeutics are delivered as prodrugs that are typically converted within the liver by cytochrome P450 (CYP) to their active forms. This conversion is often inefficient and occurs in healthy tissues away from the tumor, resulting in off target cytotoxicity (Moen et al., 2012). To address this issue CCMV ePVNs were used to encapsulate via electrostatic interactions a soluble version of a bacterial CYP (Sanchez-Sanchez et al., 2014). CCMV encapsulated CYP readily converted Resveratrol and tamoxifen prodrug formulations to their active forms and at levels comparable or greater than observed in human liver microsomal preparations. Combined with surface displayed cell targeting receptor

peptides this system provides a potential strategy to deliver nanoreactor enzymatic activities to specific cells and tissues.

Protein structural engineering methods have been applied as a means to directly convert PVN surface features into enzymatic mimics. Using *in silico* modeling methods Hou et al. identified a depression on the surface of the TMV CP permutant that resembled the catalytic site of glutathione peroxidase (GPx), an enzyme involved in oxidative protection (Dedeo et al., 2010; Hou et al., 2012). By creating substitutions at residues 142 (serine to selenocysteine) and 149 (glycine to arginine) within this catalytic site-like depression the authors were able to engineer a functional GPx reaction site on the TMV CP surface. Furthermore, in vitro assembly of the modified GPx CP produced TMV-like disks and rods displaying multiple enzyme sites with combined activities that approached that of the native GPx enzyme.

Integrated sensor systems

The ability to display selective peptides from the surfaces of ePVNs provides a unique opportunity to integrate scaffolded binding peptides into sensor systems for the detection of a range of biological and inorganic targets. One recently reported ePVN sensor system utilizes a TMV PVN system to display trinitrotoluene (TNT) binding peptides in an electrochemical detection system (Zang et al., 2014) (Fig. 4B and C). Specifically a TNT binding peptide was identified via phage display and its sequence genetically fused to the C-terminal end of the TMV CP (Jaworski et al., 2008). Solution-based TMV ePVNs displaying this TNT binding peptide were used as nano-sponges to selectively bind TNT in solution and reduce its bulk diffusion onto a sensing electrode. The measured TNT electrochemical reduction peaks were shown to correspond to the concentration of TNT. This system combines the fast response and high sensitivity of electrochemical sensing with selectivity of a scaffolded bioreceptor.

Conclusions and future directions

PVNs provide well-defined, stable and facile structures that can be readily converted into building blocks for the assembly of new nanoscale materials and devices. Structural features within the internal and external PVN surfaces are amenable to either chemical or genetic modifications for the display of novel moieties with designed functions. In addition, many of the templating systems described in this review render or produce virus particles that are non-infectious. The ability to produce such inert nanoparticles on a large agricultural/industrial scale makes plant viruses an attractive system for the production of novel renewable materials. Combined these factors have made viruses invaluable tools to investigate “bottom up” assembly approaches that have long been of interest in the engineering sciences. Yet the efforts outlined here represent only a fraction of the potential that viruses hold for nanotechnology. The majority of these studies have used PVNs as scaffolds to display, deposit or encapsulate novel functions for enhanced applications such as analyte binding or energy storage. These are static attributes and do not convey any PVN action beyond the scaffolding function. Yet, during infection virus particles function as dynamic structures capable of environmental sensing and structural alterations such as the pH derived pore openings in CCMV (Tama and Brooks, 2002). Future advances will likely develop and expand upon these dynamic biological traits to assemble multi-functional virus particles that respond to defined input signals derived from either their environment or a device interface. For example, virus structures that are able to both sense the presence of a target and then deliver a cargo will represent new theranostics tools for both threat surveillance and therapy delivery. Virus based toolboxes that utilize novel interconnects between individual particles will be used to build self-assembling 3-D meso and microscale structures much like DNA origami is currently used to fold DNA into defined nanoscale structures. And finally, artificial virus particles modeled or templated directly from their biological counterparts will be assembled from inert polymers and inorganics to function under the extreme conditions commonly produced in synthetic materials processing, thus allowing these virus-based structures to be fully integrated into traditional “top down” fabrication schemes. These are just a few of the possible advances that are likely from the emerging application of PVNs in nanotechnology.

Acknowledgments

We regret that due to space constraints we have not been able cite all of the published works that underlie or are ongoing in this field. Our own virus based nanotechnology studies are supported by the Army Research Office Biochemistry Program (Grant no. W911NF1110138) and National Science Foundation Nanomanufacturing Program (Grant no. NSF-CMMI 0927693).

References

Alonso, J.M., Gorzny, M.L., Bittner, A.M., 2013a. The physics of tobacco mosaic virus and virus-based devices in biotechnology. Trends Biotechnol. 31, 530–538.
Alonso, J.M., Ondarcuhu, T., Bittner, A.M., 2013b. Integration of plant viruses in electron beam lithography nanostructures. Nanotechnology 24.
Balci, S., Bittner, A.M., Hahn, K., Scheu, C., Knez, M., Kadri, A., Wege, C., Jeske, H., Kern, K., 2006. Copper nanowires within the central channel of tobacco mosaic virus particles. Electrochim. Acta 51, 6251–6257.
Balci, S., Leinberger, D.M., Knez, M., Bittner, A.M., Boes, F., Kadri, A., Wege, C., Jeske, H., Kern, K., 2008. Printing and aligning mesoscale patterns of Tobacco mosaic virus on surfaces. Adv. Mater. 20, 2195.
Barick, K.C., Bahadur, D., 2010. Self-assembly of colloidal nanoscale particles: fabrication, properties and applications. J. Nanosci. Nanotechnol. 10, 668–689.
Basnayake, V.R., Sit, T.L., Lommel, S.A., 2009. The Red clover necrotic mosaic virus origin of assembly is delimited to the RNA-2 trans-activator. Virology 384, 169–178.
Bittner, A.M., 2009. Electroless deposition approaching the molecular scale, Advanced Nanoscale Ulsi Interconnects: Fundamentals and Applications, pp. 221–235.
Blum, A.S., Soto, C.M., Wilson, C.D., Brower, T.L., Pollack, S.K., Schull, T.L., Chatterji, A., Lin, T.W., Johnson, J.E., Amsinck, C., Franzon, P., Shashidhar, R., Ratna, B.R., 2005. An engineered virus as a scaffold for three-dimensional self-assembly on the nanoscale. Small 1, 702–706.
Blum, A.S., Soto, C.M., Wilson, C.D., Cole, J.D., Kim, M., Gnade, B., Chatterji, A., Ochoa, W.F., Lin, T.W., Johnson, J.E., Ratna, B.R., 2004. Cowpea mosaic virus as a scaffold for 3-D patterning of gold nanoparticles. Nano Lett. 4, 867–870.
Boukamp, B.A., Lesh, G.C., Huggins, R.A., 1981. All-solid lithium electrodes with mixed-conductor matrix. J. Electrochem. Soc. 128, 725–729.
Brown, A.D., Naves, L., Wang, X., Ghodssi, R., Culver, J.N., 2013. Carboxylate-directed in vivo assembly of virus-like nanorods and tubes for the display of functional peptides and residues. Biomacromolecules 14, 3123–3129.
Browne, W.R., Feringa, B.L., 2006. Making molecular machines work. Nat. Nanotechnol. 1, 25–35.
Bruckman, M.A., Jiang, K., Simpson, E.J., Randolph, L.N., Luyt, L.G., Yu, X., Steinmetz, N.F., 2014. Dual-modal magnetic resonance and fluorescence imaging of atherosclerotic plaques in vivo using VCAM-1 targeted Tobacco mosaic virus. Nano Lett. 14, 1551–1558.
Bruckman, M.A., Soto, C.M., McDowell, H., Liu, J.L., Ratna, B.R., Korpany, K.V., Zahr, O. K., Blum, A.S., 2011. Role of hexahistidine in directed nanoassemblies of tobacco mosaic virus coat protein. ACS Nano 5, 1606–1616.
Brumfield, S., Willits, D., Tang, L., Johnson, J.E., Douglas, T., Young, M., 2004. Heterologous expression of the modified coat protein of Cowpea chlorotic mottle bromovirus results in the assembly of protein cages with altered architectures and function. J. Gen. Virol. 85, 1049–1053.
Butler, P.J.G., 1999. Self-assembly of tobacco mosaic virus: the role of an intermediate aggregate in generating both specificity and speed. Philos. Trans. R. Soc. B 354, 537–550.
Cao, J., Guenther, R.H., Sit, T.L., Opperman, C.H., Lommel, S.A., Willoughby, J.A., 2014. Loading and release mechanism of red clover necrotic mosaic virus derived plant viral nanoparticles for drug delivery of doxorubicin. Small.
Chan, C.K., Peng, H.L., Liu, G., McIlwrath, K., Zhang, X.F., Huggins, R.A., Cui, Y., 2008. High-performance lithium battery anodes using silicon nanowires. Nat. Nanotechnol. 3, 31–35.

Chapman, S., Kavanagh, T., Baulcombe, D., 1992. Potato virus-X as a vector for gene-expression in plants. Plant J. 2, 549–557.
Chen, X.L., Gerasopoulos, K., Guo, J.C., Brown, A., Ghodssi, R., Culver, J.N., Wang, C.S., 2011a. High rate performance of virus enabled 3D n-type Si anodes for lithium-ion batteries. Electrochim. Acta 56, 5210–5213.
Chen, X.L., Gerasopoulos, K., Guo, J.C., Brown, A., Wang, C.S., Ghodssi, R., Culver, J.N., 2010. Virus-enabled silicon anode for lithium-ion batteries. ACS Nano 4, 5366–5372.
Chen, X.L., Gerasopoulos, K., Guo, J.C., Brown, A., Wang, C.S., Ghodssi, R., Culver, J.N., 2011b. A patterned 3D silicon anode fabricated by electrodeposition on a virus-structured current collector. Adv. Funct. Mater. 21, 380–387.
Chen, X.L., Guo, J.C., Gerasopoulos, K., Langrock, A., Brown, A., Ghodssi, R., Culver, J.N., Wang, C.S., 2012. 3D tin anodes prepared by electrodeposition on a virus scaffold. J. Power Sources 211, 129–132.
Chiang, C.Y., Epstein, J., Brown, A., Munday, J.N., Culver, J.N., Ehrman, S., 2012. Biological templates for antireflective current collectors for photoelectrochemical cell applications. Nano Lett. 12, 6005–6011.
Comellas-Aragones, M., Engelkamp, H., Claessen, V.I., Sommerdijk, N.A., Rowan, A.E., Christianen, P.C., Maan, J.C., Verduin, B.J., Cornelissen, J.J., Nolte, R.J., 2007. A virus-based single-enzyme nanoreactor. Nat. Nanotechnol. 2, 635–639.
Correia, J.J., Shire, S., Yphantis, D.A., Schuster, T.M., 1985. Sedimentation equilibrium measurements of the intermediate-size tobacco mosaic virus protein polymers. Biochemistry 24, 3292–3297.
Cruz, S.S., Chapman, S., Roberts, A.G., Roberts, I.M., Prior, D.A.M., Oparka, K.J., 1996. Assembly and movement of a plant virus carrying a green fluorescent protein overcoat. Proc. Natl. Acad. Sci. USA 93, 6286–6290.
Dawson, W.O., Beck, D.L., Knorr, D.A., Grantham, G.L., 1986. cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcripts. Proc. Natl. Acad. Sci. USA 83, 1832–1836.
Dedeo, M.T., Duderstadt, K.E., Berger, J.M., Francis, M.B., 2010. Nanoscale protein assemblies from a circular permutant of the tobacco mosaic virus. Nano Lett. 10, 181–186.
Dedeo, M.T., Finley, D.T., Francis, M.B., 2011. Viral capsids as self-assembling templates for new materials. Prog. Mol. Biol. Transl. Sci. 103, 353–392.
Douglas, T., Strable, E., Willits, D., Aitouchen, A., Libera, M., Young, M., 2002. Protein engineering of a viral cage for constrained nanomaterials synthesis. Adv. Mater. 14, 415–418.
Douglas, T., Young, M., 1998. Host guest encapsulation of materials by assembled virus protein cages. Nature 393, 152–155.
Douglas, T., Young, M., 1999. Virus particles as templates for materials synthesis. Adv. Mater. 11, 679–681.
Dujardin, E., Peet, C., Stubbs, G., Culver, J.N., Mann, S., 2003. Organization of metallic nanoparticles using tobacco mosaic virus templates. Nano Lett. 3, 413–417.
Durham, A., Finch, J., Klug, A., 1971. States of aggregation of tobacco mosaic virus. Nat. New Biol. 229, 37–42.
Eber, F.J., Eiben, S., Jeske, H., Wege, C., 2015. RNA-controlled assembly of tobacco mosaic virus-derived complex structures: from nanoboomerangs to tetrapods. Nanoscale 7, 344–355.
Eggen, R., Verver, J., Wellink, J., De Jong, A., Goldbach, R., van Kammen, A., 1989. Improvements of the infectivity of in vitro transcripts from cloned cowpea mosaic virus cDNA: impact of terminal nucleotide sequences. Virology 173, 447–455.
Endo, M., Fujitsuka, M., Majima, T., 2007. Porphyrin light-harvesting arrays constructed in the recombinant tobacco mosaic virus scaffold. Chem.-Eur. J. 13, 8660–8666.
Evans, D.J., 2008. The bionanoscience of plant viruses: templates and synthons for new materials. J. Mater. Chem. 18, 3746–3754.
Fan, X.Z., Pomerantseva, E., Gnerlich, M., Brown, A., Gerasopoulos, K., McCarthy, M., Culver, J., Ghodssi, R., 2013. Tobacco mosaic virus: a biological building block for micro/nano/bio systems. J. Vac. Sci. Technol. A 31.
Flynn, C.E., Lee, S.W., Peelle, B.R., Belcher, A.M., 2003. Viruses as vehicles for growth, organization and assembly of materials. Acta Mater. 51, 5867–5880.
Fowler, C.E., Shenton, W., Stubbs, G., Mann, S., 2001. Tobacco mosaic virus liquid crystals as templates for the interior design of silica mesophases and nanoparticles. Adv. Mater. 13, 1266–1269.
Franzen, S., Lommel, S.A., 2009. Targeting cancer with 'smart bombs': equipping plant virus nanoparticles for a 'seek and destroy' mission. Nanomedicine-UK 4, 575–588.
Frolova, O.Y., Petrunia, I.V., Komarova, T.V., Kosorukov, V.S., Sheval, E.V., Gleba, Y.Y., Dorokhov, Y.L., 2010. Trastuzumab-binding peptide display by Tobacco mosaic virus. Virology 407, 7–13.
Gerasopoulos, K., McCarthy, M., Banerjee, P., Fan, X., Culver, J.N., Ghodssi, R., 2010. Biofabrication methods for the patterned assembly and synthesis of viral nanotemplates. Nanotechnology 21.
Gerasopoulos, K., McCarthy, M., Royston, E., Culver, J.N., Ghodssi, R., 2008. Nanostructured nickel electrodes using the Tobacco mosaic virus for micro-battery applications. J. Micromech. Microeng. 18.
Gerasopoulos, K., Pomerantseva, E., McCarthy, M., Brown, A., Wang, C.S., Culver, J., Ghodssi, R., 2012. Hierarchical three-dimensional microbattery electrodes combining bottom-up self-assembly and top-down micromachining. ACS Nano 6, 6422–6432.
Ghosh, A., Guo, J.C., Brown, A.D., Royston, E., Wang, C.S., Kofinas, P., Culver, J.N., 2012. Virus-assembled flexible electrode-electrolyte interfaces for enhanced polymer-based battery applications. J. Nanomater., 795892.
Holder, P.G., Finley, D.T., Stephanopoulos, N., Walton, R., Clark, D.S., Francis, M.B., 2010. Dramatic thermal stability of virus-polymer conjugates in hydrophobic solvents. Langmuir 26, 17383–17388.
Honarbakhsh, S., Guenther, R.H., Willoughby, J.A., Lommel, S.A., Pourdeyhimi, B., 2013. Polymeric systems incorporating plant viral nanoparticles for tailored release of therapeutics. Adv. Healthc. Mater. 2, 1001–1007.
Hou, C.X., Luo, Q., Liu, J.L., Miao, L., Zhang, C.Q., Gao, Y.Z., Zhang, X.Y., Xu, J.Y., Dong, Z.Y., Liu, J.Q., 2012. Construction of GPx active centers on natural protein nanodisk/nanotube: a new way to develop artificial nanoenzyme. ACS Nano 6, 8692–8701.
Hwang, D.J., Roberts, I.M., Wilson, T.M., 1994. Expression of tobacco mosaic virus coat protein and assembly of pseudovirus particles in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 91, 9067–9071.
Jaworski, J.W., Raorane, D., Huh, J.H., Majumdar, A., Lee, S.W., 2008. Evolutionary screening of biomimetic coatings for selective detection of explosives. Langmuir 24, 4938–4943.
Jung, S., Yi, H., 2014. An integrated approach for enhanced protein conjugation and capture with viral nanotemplates and hydrogel microparticle platforms via rapid bioorthogonal reactions. Langmuir 30, 7762–7770.
Kaur, G., Valarmathi, M.T., Potts, J.D., Jabbari, E., Sabo-Attwood, T., Wang, Q., 2010a. Regulation of osteogenic differentiation of rat bone marrow stromal cells on 2D nanorod substrates. Biomaterials 31, 1732–1741.
Kaur, G., Wang, C., Sun, J.A., Wang, Q.A., 2010b. The synergistic effects of multivalent ligand display and nanotopography on osteogenic differentiation of rat bone marrow stem cells. Biomaterials 31, 5813–5824.
Kendall, A., Bian, W., Maris, A., Azzo, C., Groom, J., Williams, D., Shi, J., Stewart, P.L., Wall, J.S., Stubbs, G., 2013. A common structure for the potexviruses. Virology 436, 173–178.
Klem, M.T., Young, M., Douglas, T., 2005. Biomimetic magnetic nanoparticles. Mater. Today 8, 28–37.
Klug, A., 1999. The tobacco mosaic virus particle: structure and assembly. Philos. Trans. R. Soc. B 354, 531–535.
Knez, M., Bittner, A.M., Boes, F., Wege, C., Jeske, H., Maiss, E., Kern, K., 2003. Biotemplate synthesis of 3-nm nickel and cobalt nanowires. Nano Lett. 3, 1079–1082.
Knez, M., Sumser, M., Bittner, A.M., Wege, C., Jeske, H., Martin, T.P., Kern, K., 2004. Spatially selective nucleation of metal clusters on the tobacco mosaic virus. Adv. Funct. Mater. 14, 116–124.
Kobayashi, M., Seki, M., Tabata, H., Watanabe, Y., Yamashita, I., 2010. Fabrication of aligned magnetic nanoparticles using tobamoviruses. Nano Lett. 10, 773–776.
Korten, T., Mansson, A., Diez, S., 2010. Towards the application of cytoskeletal motor proteins in molecular detection and diagnostic devices. Curr. Opin. Biotechnol. 21, 477–488.
Kostiainen, M.A., Hiekkataipale, P., Laiho, A., Lemieux, V., Seitsonen, J., Ruokolainen, J., Ceci, P., 2013. Electrostatic assembly of binary nanoparticle superlattices using protein cages. Nat. Nanotechnol. 8, 52 -+.
Kumar, A., Reddy, V.S., Yusibov, V., Chipman, P.R., Hata, Y., Fita, I., Fukuyama, K., Rossmann, M.G., LoeschFries, L.S., Baker, T.S., Johnson, J.E., 1997. The structure of alfalfa mosaic virus capsid protein assembled as a $T=1$ icosahedral particle at 4.0-angstrom resolution. J. Virol. 71, 7911–7916.
Lee, K.L., Uhde-Holzem, K., Fischer, R., Commandeur, U., Steinmetz, N.F., 2014. Genetic engineering and chemical conjugation of potato virus X. Virus Hybrids as Nanomaterials: Methods and Protocols, 1108; , pp. 3–21.
Lee, S.Y., Choi, J., Royston, E., Janes, D.B., Culver, J.N., Harris, M.T., 2006. Deposition of platinum clusters on surface-modified Tobacco mosaic virus. J. Nanosci. Nanotechnol. 6, 974–981.
Lee, S.Y., Royston, E., Culver, J.N., Harris, M.T., 2005. Improved metal cluster deposition on a genetically engineered tobacco mosaic virus template. Nanotechnology 16, S435–S441.
Li, F., Wang, Q.B., 2014. Fabrication of nanoarchitectures templated by virus-based nanoparticles: strategies and applications. Small 10, 230–245.
Lim, J.S., Kim, S.M., Lee, S.Y., Stach, E.A., Culver, J.N., Harris, M.T., 2010. Biotemplated aqueous-phase palladium crystallization in the absence of external reducing agents. Nano Lett. 10, 3863–3867.
Lin, T.W., Chen, Z.G., Usha, R., Stauffacher, C.V., Dai, J.B., Schmidt, T., Johnson, J.E., 1999. The refined crystal structure of cowpea mosaic virus at 2.8 angstrom resolution. Virology 265, 20–34.
Liu, L., Lomonossoff, G.P., 2002. Agroinfection as a rapid method for propagating Cowpea mosaic virus-based constructs. J. Virol. Methods 105, 343–348.
Liu, Y., Kim, E., Ghodssi, R., Rubloff, G.W., Culver, J.N., Bentley, W.E., Payne, G.F., 2010. Biofabrication to build the biology-device interface. Biofabrication 2.
Liu, Z., Qiao, J., Niu, Z., Wang, Q., 2012. Natural supramolecular building blocks: from virus coat proteins to viral nanoparticles. Chem. Soc. Rev. 41, 6178–6194.
Lomonossoff, G.P., Evans, D.J., 2014. Applications of plant viruses in bionanotechnology. Curr. Top. Microbiol. Immunol. 375, 61–87.
Loo, L., Guenther, R.H., Lommel, S.A., Franzen, S., 2007. Encapsidation of nanoparticles by red clover necrotic mosaic virus. J. Am. Chem. Soc. 129, 11111–11117.
Loo, L., Guenther, R.H., Lommel, S.A., Franzen, S., 2008. Infusion of dye molecules into Red clover necrotic mosaic virus. Chem. Commun. (Camb.), 88–90.
Lu, B., Stubbs, G., Culver, J.N., 1996. Carboxylate interactions involved in the disassembly of tobacco mosaic tobamovirus. Virology 225, 11–20.
Lu, B., Taraporewala, Z.F., Stubbs, G., Culver, J.N., 1998. Intersubunit interactions allowing a carboxylate mutant coat protein to inhibit tobamovirus disassembly. Virology 244, 13–19.
Manocchi, A.K., Seifert, S., Lee, B., Yi, H., 2011. In situ small-angle X-ray scattering analysis of palladium nanoparticle growth on tobacco mosaic virus nanotemplates. Langmuir 27, 7052–7058.
Martin, S.L., Guenther, R.H., Sit, T.L., Swartz, P.D., Meilleur, F., Lommel, S.A., Rose, R. B., 2010. Crystallization and preliminary X-ray diffraction analysis of red clover

necrotic mosaic virus. Acta Crystallogr. Sect. F Struct. Biol. Crys.t Commun. 66, 1458–1462.
McCarthy, M., Gerasopoulos, K., Enright, R., Culver, J.N., Ghodssi, R., Wang, E.N., 2012. Biotemplated hierarchical surfaces and the role of dual length scales on the repellency of impacting droplets. Appl. Phys. Lett. 100.
Miller, R.A., Presley, A.D., Francis, M.B., 2007. Self-assembling light-harvesting systems from synthetically modified tobacco mosaic virus coat proteins. J. Am. Chem. Soc. 129, 3104–3109.
Miller, R.A., Stephanopoulos, N., McFarland, J.M., Rosko, A.S., Geissler, P.L., Francis, M.B., 2010. Impact of assembly state on the defect tolerance of TMV-based light harvesting arrays. J. Am. Chem. Soc. 132, 6068–6074.
Minten, I.J., Claessen, V.I., Blank, K., Rowan, A.E., Nolte, R.J.M., Cornelissen, J.J.L.M., 2011. Catalytic capsids: the art of confinement. Chem. Sci. 2, 358–362.
Moen, E.L., Godley, L.A., Zhang, W., Dolan, M.E., 2012. Pharmacogenomics of chemotherapeutic susceptibility and toxicity. Genome Med. 4, 90.
Montague, N.P., Thuenemann, E.C., Saxena, P., Saunders, K., Lenzi, P., Lomonossoff, G.P., 2011. Recent advances of cowpea mosaic virus-based particle technology. Hum. Vaccin. 7, 383–390.
Mueller, A., Eber, F.J., Azucena, C., Petershans, A., Bittner, A.M., Gliemann, H., Jeske, H., Wege, C., 2011. Inducible site-selective bottom-up assembly of virus-derived nanotube arrays on RNA-equipped wafers. ACS Nano 5, 4512–4520.
Namba, K., Pattanayek, R., Stubbs, G., 1989. Visualization of protein-nucleic acid interactions in a virus - refined structure of intact tobacco mosaic virus at 2.9 A resolution by X-ray fiber diffraction. J. Mol. Biol. 208, 307–325.
Ng, B.C., Chan, S.T., Lin, J., Tolbert, S.H., 2011. Using polymer conformation to control architecture in semiconducting polymer/viral capsid assemblies. ACS Nano 5, 7730–7738.
Niu, Z., Bruckman, M., Kotakadi, V.S., He, J., Emrick, T., Russell, T.P., Yang, L., Wang, Q., 2006. Study and characterization of tobacco mosaic virus head-to-tail assembly assisted by aniline polymerization. Chem. Commun. (Camb.), 3019–3021.
Niu, Z., Liu, J., Lee, L.A., Bruckman, M.A., Zhao, D., Koley, G., Wang, Q., 2007a. Biological templated synthesis of water-soluble conductive polymeric nanowires. Nano Lett. 7, 3729–3733.
Niu, Z.W., Bruckman, M.A., Li, S.Q., Lee, L.A., Lee, B., Pingali, S.V., Thiyagarajan, P., Wang, Q., 2007b. Assembly of tobacco mosaic virus into fibrous and macroscopic bundled arrays mediated by surface aniline polymerization. Langmuir 23, 6719–6724.
Pogue, G.P., Lindbo, J.A., Garger, S.J., Fitzmaurice, W.P., 2002. Making an ally from an enemy: plant virology and the new agriculture. Annu. Rev. Phytopathol. 40, 45–74.
Pokorski, J.K., Steinmetz, N.F., 2011. The art of engineering viral nanoparticles. Mol. Pharm. 8, 29–43.
Pomerantseva, E., Gerasopoulos, K., Chen, X.Y., Rubloff, G., Ghodssi, R., 2012. Electrochemical performance of the nanostructured biotemplated V2O5 cathode for lithium-ion batteries. J. Power Sources 206, 282–287.
Rahman, M.M., Ölçeroğlu, E., McCarthy, M., 2014. Scalable nanomanufacturing of virus-templated coatings for enhanced boiling. Adv. Mater. Interfaces 1, 1300107–1300113.
Rong, J.H., Niu, Z.W., Lee, L.A., Wang, Q., 2011. Self-assembly of viral particles. Curr. Opin. Colloid Interface Sci. 16, 441–450.
Rong, J.H., Oberbeck, F., Wang, X.N., Li, X.D., Oxsher, J., Niu, Z.W., Wang, Q., 2009. Tobacco mosaic virus templated synthesis of one dimensional inorganic-polymer hybrid fibres. J. Mater. Chem. 19, 2841–2845.
Rothemund, P.W., 2006. Folding DNA to create nanoscale shapes and patterns. Nature 440, 297–302.
Royston, E., Ghosh, A., Kofinas, P., Harris, M.T., Culver, J.N., 2008. Self-assembly of virus-structured high surface area nanomaterials and their application as battery electrodes. Langmuir 24, 906–912.
Royston, E.S., Brown, A.D., Harris, M.T., Culver, J.N., 2009. Preparation of silica stabilized Tobacco mosaic virus templates for the production of metal and layered nanoparticles. J. Colloid Interface Sci. 332, 402–407.
Sainsbury, F., Canizares, M.C., Lomonossoff, G.P., 2010. Cowpea mosaic virus: the plant virus-based biotechnology workhorse. Annu. Rev. Phytopathol. 48, 437–455.
Sainsbury, F., Lomonossoff, G.P., 2008. Extremely high-level and rapid transient protein production in plants without the use of viral replication. Plant Physiol. 148, 1212–1218.
Sanchez-Sanchez, L., Cadena-Nava, R.D., Palomares, L.A., Ruiz-Garcia, J., Koay, M.S., Cornelissen, J.J., Vazquez-Duhalt, R., 2014. Chemotherapy pro-drug activation by biocatalytic virus-like nanoparticles containing cytochrome P450. Enzyme Microb. Technol. 60, 24–31.
Saunders, K., Lomonossoff, G.P., 2013. Exploiting plant virus-derived components to achieve in planta expression and for templates for synthetic biology applications. New Phytol. 200, 16–26.
Saunders, K., Sainsbury, F., Lomonossoff, G.P., 2009. Efficient generation of cowpea mosaic virus empty virus-like particles by the proteolytic processing of precursors in insect cells and plants. Virology 393, 329–337.
Schlick, T.L., Ding, Z.B., Kovacs, E.W., Francis, M.B., 2005. Dual-surface modification of the tobacco mosaic virus. J. Am. Chem. Soc. 127, 3718–3723.
Shenton, W., Douglas, T., Young, M., Stubbs, G., Mann, S., 1999. Inorganic-organic nanotube composites from template mineralization of tobacco mosaic virus. Adv. Mater. 11, 253.
Sherman, M.B., Guenther, R.H., Tama, F., Sit, T.L., Brooks, C.L., Mikhailov, A.M., Orlova, E.V., Baker, T.S., Lommel, S.A., 2006. Removal of divalent cations induces structural transitions in red clover necrotic mosaic virus, revealing a potential mechanism for RNA release. J. Virol. 80, 10395–10406.
Sirotkin, S., Mermet, A., Bergoin, M., Ward, V., Van Etten, J.L., 2014. Viruses as nanoparticles: structure versus collective dynamics. Phys. Rev. E 90.
Sitasuwan, P., Lee, L.A., Bo, P., Davis, E.N., Lin, Y., Wang, Q., 2012. A plant virus substrate induces early upregulation of BMP2 for rapid bone formation. Integr. Biol.-UK 4, 651–660.
Sitasuwan, P., Lee, L.A., Li, K., Nguyen, H.G., Wang, Q., 2014. RGD-conjugated rod-like viral nanoparticles on 2D scaffold improve bone differentiation of mesenchymal stem cells. Front. Chem. 2, 31.
Speir, J.A., Munshi, S., Wang, G., Baker, T.S., Johnson, J.E., 1995a. Structures of the native and swollen forms of cowpea chlorotic mottle virus determined by X-ray crystallography and cryo-electron microscopy. Structure 3, 63–78.
Speir, J.A., Munshi, S., Wang, G.J., Baker, T.S., Johnson, J.E., 1995b. Structures of the native and swollen forms of cowpea chlorotic mottle virus determined by X-ray crystallography and cryoelectron microscopy. Structure 3, 63–78.
Steckert, J.J., Schuster, T.M., 1982. Sequence specificity of trinucleoside diphosphate binding to polymerized tobacco mosaic virus protein. Nature 299, 32–36.
Steinmetz, N.F., Calder, G., Lomonossoff, G.P., Evans, D.J., 2006. Plant viral capsids as nanobuilding blocks: construction of arrays on solid supports. Langmuir 22, 10032–10037.
Steinmetz, N.F., Lin, T., Lomonossoff, G.P., Johnson, J.E., 2009. Structure-based engineering of an icosahedral virus for nanomedicine and nanotechnology. Curr. Top. Microbiol. 327, 23–58.
Stephanopoulos, N., Francis, M.B., 2011. Choosing an effective protein bioconjugation strategy. Nat. Chem. Biol. 7, 876–884.
Stock, D., Namba, K., Lee, L.K., 2012. Nanorotors and self-assembling macromolecular machines: the torque ring of the bacterial flagellar motor. Curr. Opin. Biotechnol. 23, 545–554.
Strable, E., Finn, M.G., 2009. Chemical modification of viruses and virus-like particles. Curr. Top. Microbiol. 327, 1–21.
Suci, P.A., Klem, M.T., Arce, F.T., Douglas, T., Young, M., 2006. Assembly of multilayer films incorporating a viral protein cage architecture. Langmuir 22, 8891–8896.
Tama, F., Brooks, C.L., 2002. The mechanism and pathway of pH induced swelling in cowpea chlorotic mottle virus. J. Mol. Biol. 318, 733–747.
Tilsner, J., Linnik, O., Louveaux, M., Roberts, I.M., Chapman, S.N., Oparka, K.J., 2013. Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. J. Cell Biol. 201, 981–995.
Turpen, T.H., 1999. Tobacco mosaic virus and the virescence of biotechnology. Philos. Trans. R. Soc. Lond. B Biol. Sci. 354, 665–673.
Wang, Q., Lin, T.W., Tang, L., Johnson, J.E., Finn, M.G., 2002. Icosahedral virus particles as addressable nanoscale building blocks. Angew. Chem. Int. Ed. 41, 459–462.
Wen, A.M., Rambhia, P.H., French, R.H., Steinmetz, N.F., 2013. Design rules for nanomedical engineering: from physical virology to the applications of virus-based materials in medicine. J. Biol. Phys. 39, 301–325.
Werner, S., Marillonnet, S., Hause, G., Klimyuk, V., Gleba, Y., 2006. Immunoabsorbent nanoparticles based on a tobamovirus displaying protein A. Proc. Natl. Acad. Sci. USA 103, 17678–17683.
Whaley, S.R., English, D.S., Hu, E.L., Barbara, P.F., Belcher, A.M., 2000. Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. Nature 405, 665–668.
Wilson, T.M., 1984. Cotranslational disassembly of tobacco mosaic virus in vitro. Virology 137, 255–265.
Xiong, Z.G., Lommel, S.A., 1991. Red clover necrotic mosaic virus infectious transcripts synthesized in vitro. Virology 182, 388–392.
Yang, C.X., Meldon, J.H., Lee, B., Yi, H., 2014. Investigation on the catalytic reduction kinetics of hexavalent chromium by viral-templated palladium nanocatalysts. Catal. Today 233, 108–116.
Yi, H., Nisar, S., Lee, S.Y., Powers, M.A., Bentley, W.E., Payne, G.F., Ghodssi, R., Rubloff, G.W., Harris, M.T., Culver, J.N., 2005. Patterned assembly of genetically modified viral nanotemplates via nucleic acid hybridization. Nano Lett. 5, 1931–1936.
Yi, H., Rubloff, G.W., Culver, J.N., 2007. TMV microarrays: hybridization-based assembly of DNA-programmed viral nanotemplates. Langmuir 23, 2663–2667.
Yildiz, I., Shukla, S., Steinmetz, N.F., 2011. Applications of viral nanoparticles in medicine. Curr. Opin. Biotechnol. 22, 901–908.
Young, M., Willits, D., Uchida, M., Douglas, T., 2008. Plant viruses as biotemplates for materials and their use in nanotechnology. Annu. Rev. Phytopathol. 46, 361–384.
Zan, X.J., Sitasuwan, P., Powell, J., Dreher, T.W., Wang, Q., 2012. Polyvalent display of RGD motifs on turnip yellow mosaic virus for enhanced stem cell adhesion and spreading. Acta Biomater. 8, 2978–2985.
Zang, F., Gerasopoulos, K., Fan, X.Z., Brown, A.D., Culver, J.N., Ghodssi, R., 2014. An electrochemical sensor for selective TNT sensing based on Tobacco mosaic virus-like particle binding agents. Chem. Commun. (Camb.) 50, 12977–12980.
Zeng, Q.B., Saha, S., Lee, L.A., Barnhill, H., Oxsher, J., Dreher, T., Wang, Q.A., 2011. Chemoselective modification of turnip yellow mosaic virus by Cu(I) catalyzed azide-alkyne 1,3-Dipolar cycloaddition reaction and its application in cell binding. Bioconjugate Chem. 22, 58–66.
Zhang, Z.C., Xu, B., Wang, X., 2014. Engineering nanointerfaces for nanocatalysis. Chem. Soc. Rev. 43, 7870–7886.
Zhou, J.C., Soto, C.M., Chen, M.S., Bruckman, M.A., Moore, M.H., Barry, E., Ratna, B.R., Pehrsson, P.E., Spies, B.R., Confer, T.S., 2012. Biotemplating rod-like viruses for the synthesis of copper nanorods and nanowires. J. Nanobiotechnol. 10, 18.
Zhou, K., Li, F., Dai, G.L., Meng, C., Wang, Q.B., 2013. Disulfide bond: dramatically enhanced assembly capability and structural stability of tobacco mosaic virus nanorods. Biomacromolecules 14, 2593–2600.